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GROWTH CURVES OF AZOTOBACTER AT DIFFERENT pH LEVELS[1]

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INTRODUCTION

Adequate data have been accumulated during the past three decades to show that the H^+ concentration of the medium in or upon which *Azotobacter* is grown exercises a marked influence upon certain of its physiological activities. Students of this organism have placed special emphasis upon the effect of the H^+ concentration 077: (1) Its distribution in soils; (2) its ability to bring about the fixation of uncombined atmospheric nitrogen; (3) its respiration; and (4) its growth.

The available data relative to be influence of the H^+ concentration upon distribution and nitrogen fixation point rather conclusively to a limiting H^+ concentration, expressed as pH, in the vicinity of 6.0 for most strains of *Azotobacter*. For nitrogen fixation Burk and his colleagues (*3*, *4*)[2] recorded the specific limiting pH value of 5.97, and suggested that at lower pH values the nitrogen-fixing enzyme is inactivated.

In view of the variability recorded for other characteristics of *Azotobacter*, it is questionable whether one should expect a single specific limiting H^+ concentration for the various physiological activities of this genus, or even a constant limiting H^+ concentration applicable to all strains for any single function. In fact, Starkey (*11*) has shown that for at least one strain or species the limiting H^+ concentration for activity in general approximates pH 3.0 instead of pH 6.0.

According to Burk and his colleagues (*2*), who have investigated the influence of the H^+ concentration upon respiration of *Azotobacter* extensively, limited temporary respiration may take place at pH levels of 5.0 or less. However, a rapid decrease in the respiration rate was recorded as the pH fell below 6.0, accompanied by a gradual inactivation of the enzyme system involved. The lower the pH level the less active and the less stable the enzyme system became.

The data relative to growth are less conclusive. Burk and his associates (*4*) observed no growth of *Azotobacter* in a medium free of combined nitrogen when the pH fell below 5.97. On the other hand, measurable growth was noted at pH values of 4.0 to 4.5 when fixed nitrogen was included in the medium. This would indicate that the processes involved in growth continued active at H^+ concentrations inhibitory to the metabolism of elementary nitrogen. This fact alone is not particularly surprising. If the nitrogen-fixing enzyme system is inactivated at a pH value of 5.97, no growth could be expected in a medium free of combined nitrogen at pH values below that point, since available nitrogen would become the limiting factor in growth.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 236.

However, if "Respiration gradually ceases or comes to an insignificant value at pH 6.0 as the result of an integrated combination of reversible and irreversible time factors" (*2, p. 438*), it is difficult to see how growth could continue below pH 5.97 even in the presence of combined nitrogen.

Furthermore, if under natural soil conditions, *Azotobacter* metabolizes only fixed nitrogen (*1*), as it apparently does in the laboratory where adequate combined nitrogen is available, it is also difficult to understand why it is not found in abundance in fertile soils with pH values below 6.0. Such soils furnish adequate quantities of combined nitrogen for the abundant growth of higher plants and saprophytic micro-organisms in general. During the growing season, such soils contain appreciable quantities of nitrate and other forms of nitrogen known to be available to *Azotobacter*, and there is no evident reason why it should not compete successfully with other soil saprophytes and higher plants for this nitrogen. The mere fact that *Azotobacter* is seldom found naturally (*9*) in such soils and disappears from them when introduced artificially (*8, 10*) is a priori evidence that growth does not take place in them. Such discrepancies suggested the desirability of further investigations relative to the influence of H^+ concentration upon the growth of *Azotobacter*.

EXPERIMENTAL PROCEDURE

Numerous preliminary experiments involving over 40 strains of *Azotobacter* were carried out on washed mannitol agar slants adjusted to different pH levels. Observations in parallel of growth on a nitrogen-free medium and in the presence of fixed nitrogen (KNO_3) were recorded by this procedure. Only freshly prepared slants were used, and uniform inoculation was obtained by employing one drop of a heavy suspension of young cells as the inoculum for each culture in any single experiment. This procedure obviously did not lend itself to accurate quantitative measurements of growth. Visual records of growth taken after 48, 96, and 168 hours gave valuable qualitative information, particularly as to the range in pH value over which subsequent studies of different strains should be conducted.

Most of the data here recorded were obtained by growing the various strains in vigorously aerated liquid media adjusted to different pH values. After suitable incubation periods quantitative measurements of turbidity, volume of cells, pH level, microscopic count of cells, and in some instances, energy material consumed, served as criteria of growth.

The medium used was the usual salts medium composed of 3.0 gm. of a mixture of KH_2PO_4 and K_2HPO_4 (the ratio depending upon the approximate pH value to which it was desired to buffer the basic medium); 0.2 gm. NaCl; 0.2 gm. $M_9SO_47H_2O$; 0.02 gm. $CaCl_2$; 0.02 gm. $CaCO_3$; 3 drops of a 10 percent soloution of $FeCl_3$; 3.0 p.p.m. MoO_3; 1,000 ml. distilled water; and 20.0 gm. mannitol or other energy material. Fixed nitrogen was supplied in the form of ammonium sulphate in a concentration of 100 p.p.m., unless otherwise stated. An ammonium salt was employed because any appreciable utilization of the ammonium ion would increase the H^+ concentration of the medium, and thus tend to inhibit rather than to stimulate growth. Two hundred milliliter quantities of the medium were sterilized in 300 milliliter pyrex Erlenmeyer flasks. Aerating tubes

inserted in nonabsorbent cotton stoppers wrapped in gauze were sterilized separately and inserted after inoculation.

The inoculum was grown on mannitol agar contained in Blake bottles usually for 18 to 24 hours or longer, depending upon the rate of growth, and suspended in sterile culture medium. Where gum formation was abundant it was found advantageous to shake the suspension of cells thoroughly in a flask containing a few sterile glass beads. Carefully measured quantities of such a suspension of cells were added to each flask in a given experiment to insure uniform inoculation. No effort was made to obtain a constant concentration of cells in the inocula employed in different experiments; hence the different experiments cannot be compared directly insofar as the initial number of cells is concerned. However, a glance at the various charts subsequently presented will show that cessation of growth was in no way related to number of cells.

Minor variations in the pH level of the culture medium were obtained by adding the necessary quantity of NaOH or HCl. Because of the difficulty in maintaining seals where pressure is employed and frequent sampling is necessary, each flask was attached separately to the compressed air system. This led to some variation in the rate of air flow through the different cultures in a given experiment, but observation has shown that where vigorous bubbling of air through cultures is maintained, variation in growth between replicated flasks is not very great. Incubation was at 28° to 32° C. Aeration for 30 minutes preceded sampling for the initial analysis, thus insuring a homogenous distribution of cells.

A Petroff-Hausser counting chamber was employed for determining the number of cells. If the concentration of cells was high, a preliminary dilution was made. Difficulty was encountered in attaining the high degree of accuracy in counting cells that has been reported for other cultures (*5*). It was therefore desirable to make a sufficient number of replicate counts to permit statistical treatment of the data. Hence, in most instances reported, the entire ruled area of five separate mounts was counted. It was impossible to complete all the other quantitative measurements and make a larger number of counts without consuming so much time that significant changes might have taken place before all analyses could be completed. The data have been subjected to Fisher's (*7*) t test for significant differences in the number of cells at the different analyses. Because of the difficulty of indicating such calculated values graphically it has been arbitrarily assumed that when the calculated t value was less than the t value in Fisher's table at the 5 percent level the increase was not significant. This has been indicated on the accompanying graphs by an arrow pointing to the last analysis beyond which no such increase was recorded.

Where active reproduction of *Azotobacter* in aerated liquid cultures is taking place, cells may be observed in all stages of division, but little tendency to adhere together after division is noted. Since the point at which a dividing cell should be regarded as two individual cells is at best arbitrary, all dividing cells were counted as single units. In spite of continuous agitation due to aeration and vigorous shaking immediately before sampling, composite counts obtained by this procedure were occasionally obviously out of line. As yet no practicable means of eliminating such errors has been devised.

Turbidity was measured with the aid of a Gates suspensiometer and readings have been recorded in the reciprocal of depth in centimeters of suspension necessary to obscure the wire loop.

The volume of cells was determined by centrifuging a measured volume of the culture in a Hopkins vaccine tube and reading the volume directly. Many of the data obtained by this method were obviously unsatisfactory. Cultures occasionally flocculated and the degree of packing appeared to be influenced also by the presence of gum which varied widely among the different strains and possibly with other factors such as age.

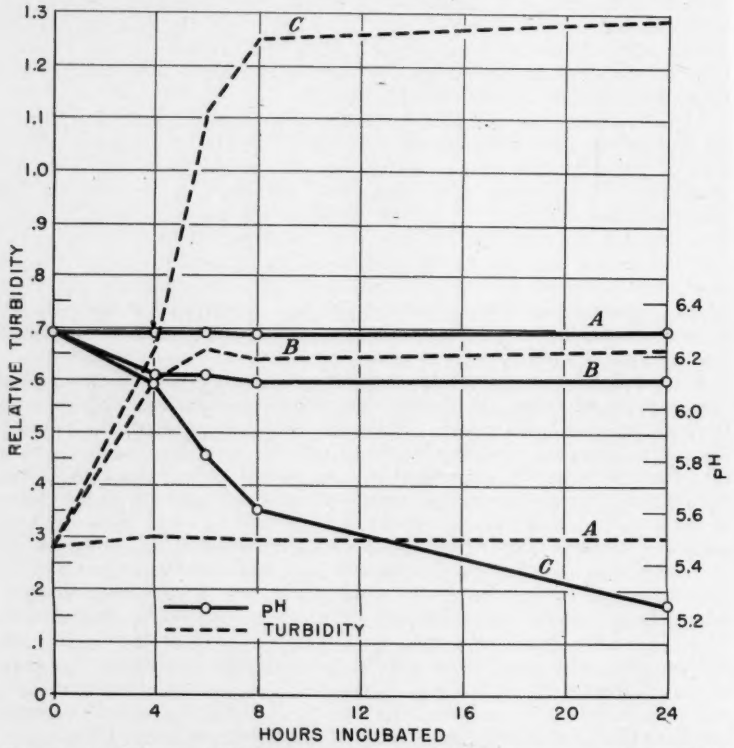

FIGURE 1.—Influence of H_9Cl_2 upon pH and turbidity curves, *Azotobacter* strain 32. *A*, H_9Cl_2 added initially; *B*, H_9Cl_2 added after 4 hours' incubation; *C*, no H_9Cl_2 added. Note constancy of pH and turbidity values in the presence of H_9Cl_2; i. e., in the absence of bacterial activity.

Hydrogen-ion concentration was measured at each analysis with the aid of a glass electrode and, as indicated in figure 1, replicated determinations made under conditions where no changes would be expected checked within a few hundredths of a pH unit even though 24 or more hours intervened between readings.

Following the final analysis, and frequently at each analysis, every culture was checked for purity by streaking on plates of both nutrient and mannitol agar and, after adequate incubation, examining the

growth carefully under the 16- and 4-mm. objectives for indications of contamination. All data obtained from cultures showing indications of contamination were discarded. Contaminants were easily detectable only when cultured on nutrient agar.

EXPERIMENTAL DATA

GROWTH ON SOLID MEDIA

In table 1 are recorded the data from two experiments in which the growth of 37 strains of *Azotobacter chroococcum* Beij. was observed on washed mannitol agar adjusted to different pH levels, one series of cultures being grown on nitrogen-free agar, the other on the same medium containing fixed nitrogen in the form of KNO_3. These two experiments were selected from among many only because they gave a better distribution of initial pH values. Growth was recorded after 2, 4, and 7 days' incubation, but the inclusion of data for the two shorter periods would add little except to indicate that frequently growth started somewhat sooner in the presence of fixed nitrogen and that near the limiting pH growth was extremely slow with all strains. In view of the ready availability of nitrate nitrogen to so many higher plants, fungi, and bacteria and the relative inertness of elementary nitrogen, the more rapid growth in the presence of the fixed nitrogen is not surprising.

These data definitely indicate that there is no significant difference in the critical H^+ concentration for the growth of *Azotobacter* in the presence or absence of fixed nitrogen, at least insofar as these 37 strains, isolated from a wide variety of soils, represent the group. Without exception, every strain grew at a pH value of 6.0 while no strain grew at a pH value of 5.39 or 5.44. An analysis of growth records at all pH values lying between 5.44 and 6.00 reveals 89 failures to grow in the absence of fixed nitrogen and 84 failures to grow in the nitrogen-containing medium. Also, there were 4 cultures for which growth was recorded at a slightly lower pH level in the nitrogen-free medium and 9 cultures in which the reverse was true.

TABLE 1.—*Growth of Azotobacter chroococcum on nitrogen-free mannitol agar and on mannitol agar containing KNO_3, at different initial pH levels*

Culture No.	Growth of *Azotobacter* on nitrogen-free agar with initial pH of—								Growth of *Azotobacter* on agar containing KNO_3 with initial pH of—							
	[1] 5.44	[1] 5.58	[1] 5.71	[2] 5.83	[2] 5.92	[2] 6.00	[2] 6.17	[1] 6.17	[1] 5.39	[1] 5.60	[1] 5.73	[2] 5.83	[2] 5.92	[2] 6.00	[2] 6.17	[1] 6.19
28	0	0	0	0	+++	+++	++++	++++	0	0	0	0	+++	++++	++++	++++
61	0	0	0	0	++	++++	++	++++	0	0	0	0	0	+	++++	++++
60	0	?	+	++	++++	++++	+++	++++	0	+	++	++++	++++	++++	++++	++++
A	0	0	0	?	++++	++++	++++	++++	0	0	0	0	++++	++++	++++	++++
O_2	0	0	0	0	+	++++	++++	++++	0	+	++	++++	++++	++++	++++	++++
17	0	+	++	++++	++++	++++	++	++++	0	0	0	0	0	++++	++++	++++
F2	0	0	0	0	++	++	++++	++++	0	0	0	0	++++	++++	++++	++++
70	0	0	0	0	++	++	++	++++	0	0	0	0	++++	++++	++++	++++
22	0	0	0	0	+++	+++	+	++++	0	0	0	0	++++	++++	++++	++++
37b	0	0	0	0	+	+	+++	++++	0	0	0	0	+	++++	++++	++++
54	0	0	0	0	+	+++	++++	++++	0	0	0	0	0	++++	++++	++++
11	0	0	0	0	0	++	++	++++	0	0	0	0	0	++++	++++	++++
44	0	0	0	0	++	++	++++	++++	0	0	0	0	+++	++++	++++	++++
12	0	0	0	0	+	++++	++++	++++	0	0	0	+	+++	++++	++++	++++
10	0	+	+	+++	++++	++++	++++	++++	0	+	0	0	++++	++++	++++	++++
21	0	0	0	0	+++	++++	++++	++++	0	0	0	0	0	+	++++	++++
37	0	0	0	0	++	++++	++++	++++	0	0	0	0	0	+	++++	++++
J4–b	0	0	0	0	0	++++	++++	++	0	0	0	0	0	+++	++++	++++
14b	0	0	0	0	+	++	++++	++++	0	0	0	0	0	+++	++++	+++
121	0	0	0	0	++	++	++++	++++	0	0	0	0	0	+++	++++	++++
50	0	?	+	+++	++++	++++	++++	++++	0	?	+	+	+++	++++	++++	++++
25	0	0	?	+++	++++	++++	++++	++++	0	0	+	+	++++	++++	++++	++++
6a	0	0	0	++	++++	++++	++++	++++	0	0	0	++	++++	++++	++++	++++
O2–b	0	0	0	0	+++	++++	++++	++++	0	0	0	0	++++	++++	++++	++++
V3	0	0	0	0	++	++++	++++	++++	0	0	0	0	0	+	++++	++++
P1	0	0	0	0	++	++++	++++	++++	0	0	0	0	0	+	++++	++++
K	0	0	0	0	++	++	++++	++++	0	0	0	0	0	+	++++	++++
32	0	++	++	+++	++++	++++	++++	++++	0	++	+++	+	++++	++++	++++	++++
24	0	++	++	++++	++++	++++	++++	++++	0	++	++	++	++++	++++	++++	++++
N_4	0	0	0	0	++	++++	++++	+++	0	0	0	0	+	+	++++	++++
33	0	+	++	+++	++++	++++	++++	++++	0	++	+++	++	++++	++++	++++	++++
14	0	0	0	0	+	++	++++	++++	0	0	0	0	+++	++++	++++	++++
13	0	0	0	0	+	+++	++++	++++	0	0	0	0	+	+++	++++	++++
118	0	0	0	0	?	+	++	++++	0	0	0	0	0	++	++++	++++
3	0	++	++	++++	++++	++++	++++	++++	0	++	++	++	++++	++++	++++	++++
67	0	0	0	0	?	+	+	++++	0	0	0	0	?	++++	++++	++++
34	0	0	++	+	++++	++++	++++	++++	0	0	0	+	+	+++	++++	++++

[1] Experiment conducted Nov. 10, 1939.
[2] Experiment conducted Dec. 2, 1939.

These data indicate some variation in the limiting H^+ concentration for growth among the different strains, the maximum variation approximating 0.5 pH unit. In the presence of fixed nitrogen, for example, 6 strains ceased growth between pH 6.0 and 5.92; 14 between pH 5.92 and 5.83; 8 between pH 5.83 and 5.73; while 9 strains exhibited more tolerance toward hydrogen ions but ceased growth altogether before pH 5.44 was reached. If the 48-hour records had been included it would be evident that growth of a dozen or more strains was seriously retarded at a pH value as high as 6.17, either a zero or + representing growth as compared with the normal ++++ for more favorable pH values.

In view of subsequent observations relative to changes in the pH value of cultures in which no growth, ie., no increase in cell numbers, took place, it is probable that some instances of growth are recorded in table 1 at pH levels where growth actually would not take place. The utilization of the NO_3 ion results in a marked elevation in pH (fig. 5, B_5). If the initial H^+ concentration happened to be only slightly above the critical point it is conceivable that the cells originally introduced as the inoculum might lower the H^+ concentration sufficiently to make some growth possible. If such is the case each successive increment of growth would render the conditions more favorable. Some evidence in support of this view has been obtained. The method of inoculation already described resulted in a massing of cells at the butt of the slant. In nearly all instances where limited growth (+) was recorded, it took place very slowly and was confined to the butt. In some instances where growth was questionable after 96 hours' incubation, a restreaking of cells from the butt of the slant resulted in good growth during the following 72 hours. This would indicate that some radical alteration had taken place either in the medium or in the organisms since the original inoculation, possibly as a result of a change in the reaction of the medium or a rather rapid adaptation of the organisms to the more acid condition. Such an adaptation does not appear to take place in soils either under natural or experimental field conditions. Neither repeated inoculation nor the constant maintenance of *Azotobacter* in adjacent soil over a period of 20 years has resulted in the establishment of strains of *Azotobacter* in soils with pH values only slightly below 6.0.

GROWTH IN LIQUID MEDIA

Because of the much more rapid growth obtainable in aerated liquid media and the possibility of applying quantitative analytical methods, it was hoped that a more accurate determination of the end point of growth could be made by the use of such methods. The general procedure followed was to start a given experiment early and make as many quantitative measurements of growth during the day and evening as possible, and to follow these with one anaylsis the next morning. Data obtained by this procedure are amenable to graphic presentation and such a method has been followed. An effort has been made to select from the mass of data accumulated during the past few years representative experiments to illustrate the major points brought out in these studies. These data are presented in figures 1 to 11. Each graph could be replicated a number of times if space permitted.

The primary objective was to determine the relationship, if any, between the H^+ concentration of the medium and growth of *Azoto-*

bacter. Since several criteria of growth were used and these were sometimes found to measure separate and not necessarily related factors, it will be helpful to indicate more precisely just what factors each criterion did evaluate.

The term "growth" as employed in bacteriologic literature implies, unless otherwise defined, an increase in the number of individual cells. If this concept of growth is adhered to, then a cell count should be the most, and possibly the only accurate, measure of growth. Because of certain inherent difficulties encountered in determining accurately the number of bacterial cells, other methods of estimating growth have been introduced. It has been assumed that these methods more or less accurately indicate the number of cells; hence may be employed in measuring growth.

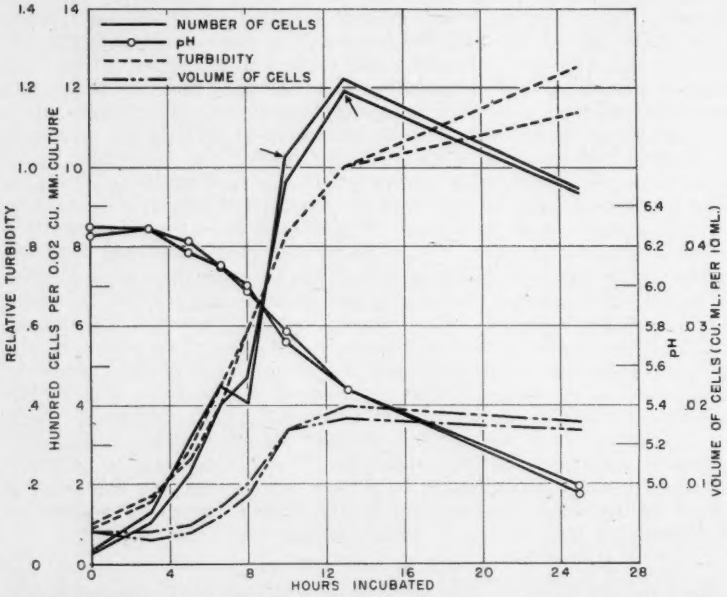

FIGURE 2.—Number of cells, pH value, turbidity, and volume of cells recorded from duplicate cultures of *Azotobacter* strain 32. Arrows indicate points beyond which gains were not significant at the 5-per cent level.

Among the indirect means employed to measure growth, turbidimetric methods rank first. The use of this procedure is predicated upon the assumption that there is a direct relation between turbidity and the number of particles (bacterial cells) in suspension, an assumption that is open to question. Turbidity results from the so called "scattering" (reflection) of light rays by the particles in suspension. Since size and composition as well as the number of suspended particles influence "scattering" phenomena, a direct relationship between turbidity and the number of suspended particles could be expected only when the particles are constant in size and composition.

As long as rapid cell division is taking place in an aerated culture of *Azotobacter*, the general appearance of the cells remains quite

similar. A glance at figures 2, 6, 8, and 9, will show that during this phase of growth the curves for turbidity and number of cells are similar. The smoother turbidity curves in these comparisons would indicate a smaller experimental error in turbidity measurements than in cell counts.

Frequent observation of aerated liquid cultures during a complete growth cycle will leave no doubt as to the occurrence of significant changes both in size and composition of the individual *Azotobacter* cell—changes equally as radical as those that have so frequently been recorded photographically for cultures grown under other conditions (e. g., photographs by den Dooren de Jong (*6*)). After reproduction ceases in aerated *Azotobacter* cultures the individual cell may continue to increase in size until it exceeds its original volume tenfold or more. Furthermore, the often recorded "increase in refractivity" or accumulation of "storage products" may also take place under these conditions to such an extent as to result in parts or even in the entire cell becoming almost opaque

Still a third factor may enter the optical picture in that some strains may bring about a marked accumulation of colloidal excretory products, so-called gums, which influence the optical properties of some cultures and not others, or the same culture at one time and not another. It is obvious from figures 2, 6, 7, and 9 that, as a result of the operation of some such factors, the turbidity curve does not necessarily follow the numbers curve after the logarithmic growth phase has been passed.

In addition to number of cells and turbidity, a third criterion was employed to measure growth in these studies, namely, the volume of cells. Obviously the actual volume of cells will depend upon the number of cells times size. Attention has just been called to the variation in size of the individual cell as influenced by reproduction and age. Another factor influencing the *apparent* volume is the extent to which packing is accomplished in making volumetric measurements. Even when the centrifugal force applied is constant, packing appears to be markedly influenced by other factors, such as gum formation and flocculation, which varied widely, not only between strains but for the same culture at different ages. The close similarity between the curves for number, turbidity, and volume in certain instances (figs. 2 and 8) indicates that volume may represent growth fairly accurately. In other instances the volume curves obviously bore little relation to growth and have been omitted.

Quantitative measurements of some byproduct of metabolic activity, such as nitrogen fixed, or of some nutritive material utilized, such as the uptake of oxygen, have been employed as indicators of growth. In these studies easily measured glucose was sometimes substituted for mannitol as the source of energy and quantitative changes in the glucose content of the cultures were recorded.

It was early noted that changes in the H^+ concentration of a culture, when the mildly buffered liquid medium employed contained ammonium sulfate, paralleled the disappearance (utilization) of the NH_4 ion. Since $pH = \log 1/H^+$ the pH curve of a culture wherein growth is taking place should be inversely related to NH_4 utilization, and hence to growth as represented by NH_4 utilization. It should be kept in mind, however, that in a nitrogen-free medium or in a medium containing fixed nitrogen in the form of KNO_3 the above

described relationship between growth and pH does not hold. In the latter case a decrease in the H^+ concentration parallels the utilization of NO_3 ions due to the excess of K ions (fig. 5).

In general the carbohydrate utilization curve, pH curve (inverted), and turbidity curve paralleled the cell-number curve so long as active cell division was taking place. After the cessation of active growth the close relationship between these various factors did not always continue. As a rule pH values continued to decrease, provided the supply of NH_3–N was not exhausted; cell counts also decreased, possibly as a result of flocculation or biochemical disintegration; and turbidity

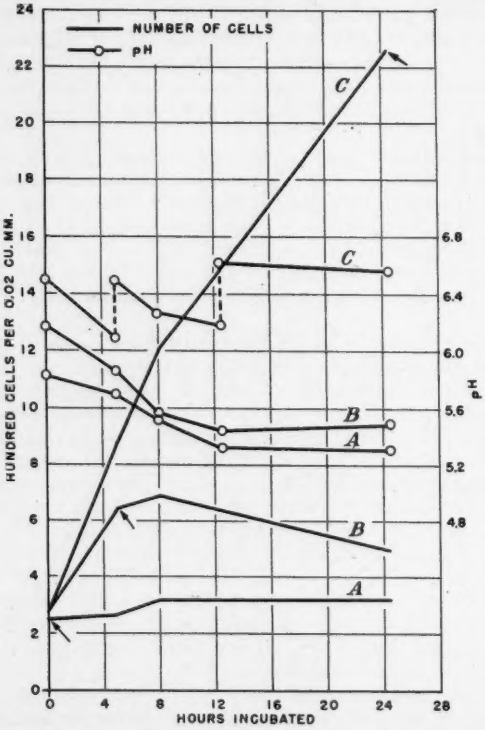

FIGURE 3.—Curves showing the influence upon growth of *Azotobacter* strain 33L of favorable pH levels maintained by neutralizing acidity with NaOH. *A*, *B*, and *C* received identical treatment except with respect to initial pH; broken portion of pH curve for *C* indicates readjustment of pH by addition of NaOH.

continued to increase. It is known from respiration studies on resting *Azotobacter* cells that many organic substrates may be utilized in appreciable quantities long after reproduction has ceased. Large quantities of glucose were observed to disappear from heavy suspensions of cells in the absence of any significant increase in the number of cells (figs. 4, A and B). This observation relative to glucose is not unexpected in view of the often recorded utilization of glucose by resting cells in respiration studies.

DISCUSSION

The data presented in table 1 indicate that the maximum H^+ concentration compatible with the growth of different strains of *Azotobacter* varies within the approximate limits, expressed as pH, of 5.5 and 6.0. However, as the maximum limiting H^+ concentration is approached growth is greatly retarded. The presence of combined nitrogen exerted no influence upon the critical H^+ concentration for growth.

The data presented graphically in figures 1 to 11 lend substantial support to a number of observations among which the following appear worthy of note.

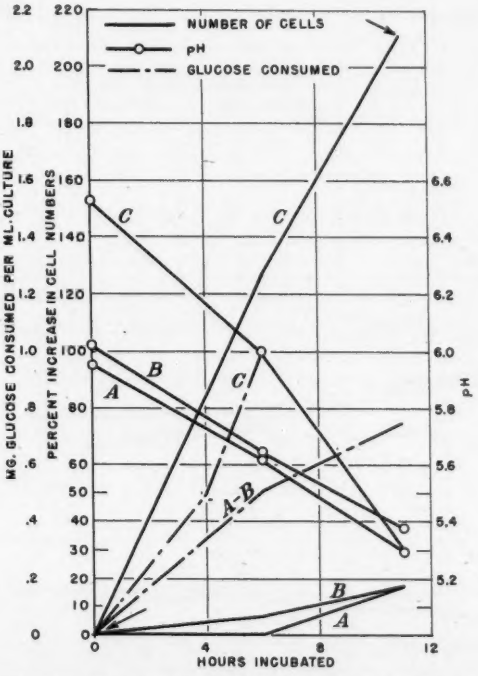

FIGURE 4.—Utilization of glucose by *Azotobacter* strain C_7b_4 in the presence of growth (*C*) and in the absence of growth (*A* and *B*) but in the presence of large numbers of cells. (Initial cell count 2,100 per 0.02 cu. mm.). Cell-number curve plotted on percentage increase in numbers.

The curves representing number of cells are characteristic of growth curves in general. In some instances, possibly because of inappropriate timing of analyses, the initial lag is not evident (figs. 3, 4, 8, and 9). The maximum number of cells was often reached in 4 to 6 hours (figs 7 and 9, *B*), and sometimes represented a very small total increase, not exceeding 5,000,000 per milliliter (fig. 5, B_2, B_3, and B_4). Retardation or cessation of active reproduction was: (1) independent of the number of cells, the maximum number of cells reached varying more than tenfold (compare curve *B* with *C* in figure 3, and curve B_5 with

B_2, B_3, and B_4 in figure 5); (2) not due to the exhaustion of the energy substrate (fig. 6); and (3) not a function of time, becoming operative within 4 to 6 hours (figs. 3, *B*; 7; and 9, *B*) or only after 48 hours (fig. 5, B_5).

Turbidity curves were of the same general form as those representing number of cells except that turbidity frequently continued to increase after cell division had ceased (figs. 6 and 7, and fig. 9, *B*) and sometimes developed in the absence of any reproduction. Quantitatively, however, the relationship between turbidity and number of cells varied for different cultures, the ratio cell numbers: turbidity being approximately seven times as great in one instance as in another. Turbidity may continue to increase for a time after cessation of re-

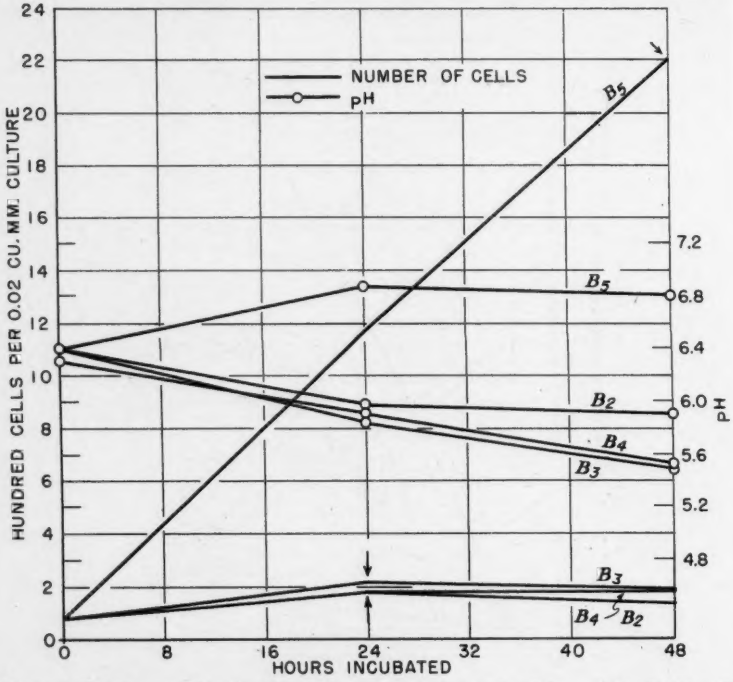

FIGURE 5.—Curves showing the influence upon growth of favorable (high) pH levels (curves B_5) maintained through absorption of the NO_3 ion when KNO_3 was present, *Azotobacter* strain 37b. B_2, B_3, and B_4 media contained $(NH_4)_2SO_4$; B_5 media contained KNO_3.

production at approximately the same rate as during the phase of rapid growth (figs. 6 and 7). This variation in relative turbidity may be explained on the basis of variation in size and composition of the cells of different strains, while the increase in turbidity in the absence of growth probably resulted from an increase in size and change in composition with increased age.

Where satisfactory volumetric measurements were obtained, curves plotted from the data were of the same general type as those for number of cells (figs. 2 and 8).

Changes in the pH value of a medium containing ammonium sulfate are inversely related to changes in cell number and turbidity, at least until active reproduction ceases (figs. 1 to 11). After the phase of active growth, however, the pH curve may continue to fall, even though the number of cells is constant or is decreasing (figs. 2; 5, B_3 and B_4; 7; and 9, *B*). The decrease in pH (increase in H^+) in such cases is due primarily to the absorption of NH_4 ions, hence is influenced by the original concentration of NH_4 (fig. 9), or by NH_4 subsequently added to the medium. Further decreases in H may cease altogether when the supply of NH_4 is exhausted (fig. 9, *A*) in spite of the fact that growth may continue. The absorption of NH_4 and the accompanying fall in pH may continue after reproduction has ceased (fig. 9, *B*) or may be markedly evident in the absence of growth provided the

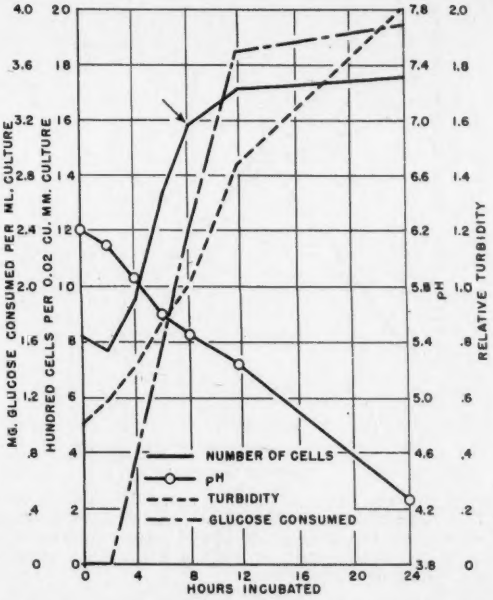

FIGURE 6.—Relation between growth and utilization of glucose by *Azotobacter* strain 13. Cessation of growth was not due to glucose exhaustion, the medium still containing 8.33 mg. of glucose per ml. of culture at the 24-hour analysis.

number of cells introduced in the initial inoculum is high enough to bring about quantitatively measurable changes (fig. 4, *A*, and *B*).

Curves based upon the quantity of energy substrate utilized tend to parallel growth curves where active reproduction is taking place (figs. 4, C, and 6); however, following a heavy inoculation under conditions unfavorable for cell division, relatively large quantities of glucose may be metabolized (fig. 4, *A* and *B*). The glucose consumption under such conditions may closely parallel the development of turbidity.

The general type of the growth curve (turbidity in this case) is similar, whether glucose, fructose, or mannitol is the source of energy (fig. 10).

As already suggested, cell-number and pH curves are inversely related during active growth. The higher the initial pH, within the limit of these studies, the higher the maximum number of cells reached (figs. 3, 4, and 11) and the longer the incubation period necessary before the maximum is reached.

If the initial H^+ concentration is above a maximum, varying between the pH values of 5.5 and 6.0 for different strains, no increase in cell numbers will take place and as the H^+ concentration approaches this maximum the rate of growth is greatly retarded (figs. 3 and 4).

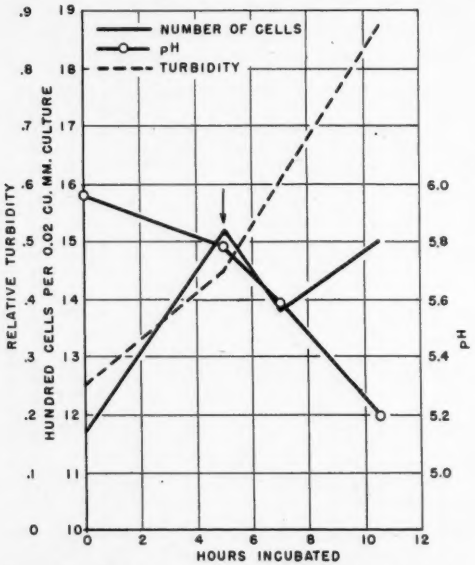

FIGURE 7.—Curves showing how cessation of growth of *Azotobacter* strain C_7–b_2 was followed by a marked increase in turbidity and utilization of fixed nitrogen. The original 10 p. p. m. of nitrogen added was exhausted after 5 hours, and an additional 5 p. p. m. disappeared during the following 2 hours without any accompanying increase in cell numbers.

If the initial H^+ concentration is such that active cell division can take place and as a result of this growth the H^+ concentration increases, as soon as it reaches the maximum for the particular strain in question further cell division will cease (figs. 1 to 11). On the other hand, if the necessary conditions exist for maintaining the H^+ concentration below the indicated maximum, growth will continue until limited by some other factor (figs. 3, 5, 9, and 11).

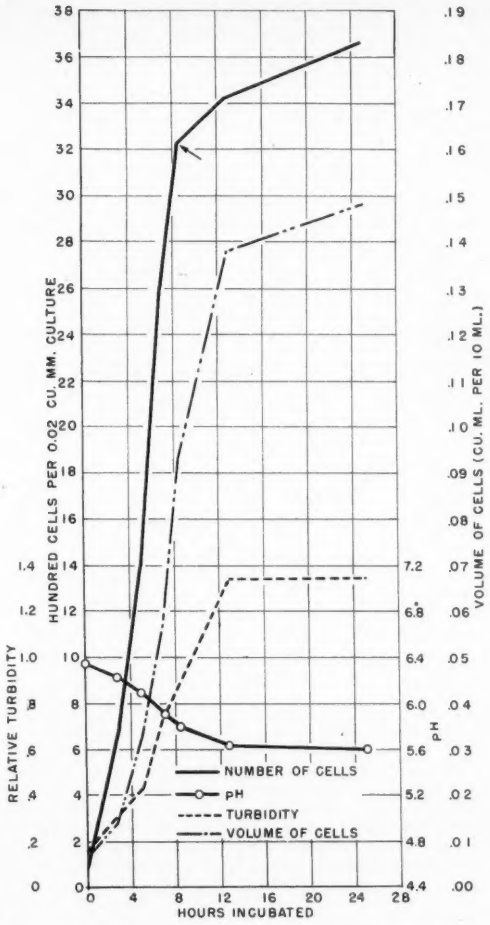

FIGURE 8.—Curves showing high bacterial numbers, *Azotobacter* strain C_7–b_3 accompanied by low turbidity.

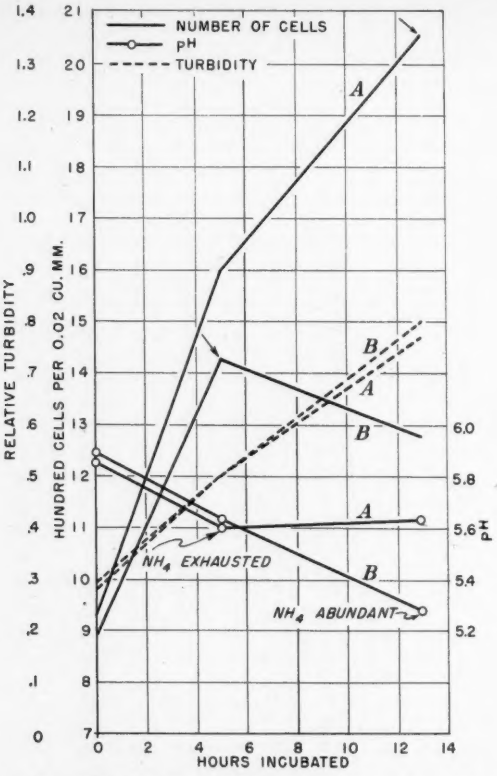

FIGURE 9.—*A* shows continued growth of *Azotobacter* after exhaustion of fixed nitrogen in medium when initial nitrogen content was 10 p. p. m. and pH was favorable. Nitrogen was exhausted at 5 hours, pH was constant thereafter, and growth continued. *B* shows how growth ceased when original nitrogen content was 25 p. p. m., nitrogen was abundant, and pH content continued to fall.

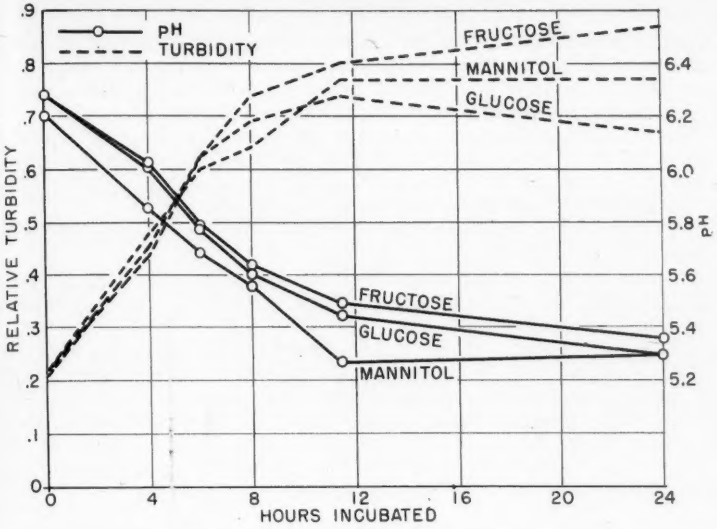

FIGURE 10.—Curves showing the influence of the source of energy upon growth (turbidity) of *Azotobacter* strain O_2.

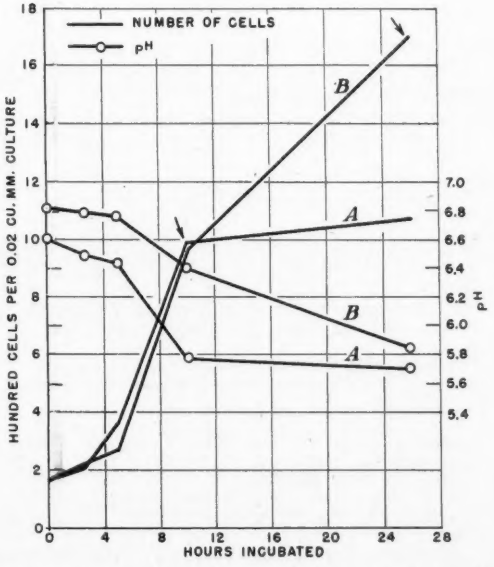

FIGURE 11.—Curves showing the influence upon growth of *Azotobacter* strain 22 of temporary maintenance of favorable pH by the addition of $CaCO_3$. The *B* culture is a duplicate of *A* except that it contained a small quantity of additional $CaCO_3$

SUMMARY

Data accumulated from an intensive study of more than 40 pure-cultures of *Azotobacter* warrant the conclusion that the H^+ concentration of the medium exercises a profound influence, either directly or indirectly, upon the growth (reproduction) of this organism.

The maximum H^+ concentration compatible with growth varies somewhat with different strains but in general falls within the limits, expressed as pH, of 5.5 and 6.0. Growth is markedly retarded within a few tenths pH unit of the critical H^+ concentration.

The presence of fixed nitrogen does not alter the critical H^+ concentration for any strain studied.

Increase in size of the individual cell and metabolization of fixed nitrogen and energy substrate can continue at H^+ concentrations inhibitory to cell division.

In a liquid medium culture of *Azotobacter*, during the period of active cell division, changes in turbidity, cell volume, energy substrate, and (in the presence of ammonium sulfate) ammonium nitrogen and H^+ concentration parallel changes in cell numbers; under such conditions, therefore, these changes may be taken as criteria of growth. In the absence of active cell division, however, any one or all of these factors may undergo radical alteration independent of growth.

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THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE SET OF PODS AND YIELD OF WHITE PEA BEANS [1]

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INTRODUCTION

Approximately 87 percent of all white pea beans (*Phaseolus vulgaris* L.) grown in the United States are produced in Michigan, and 90 percent of the total acreage in Michigan is in 19 counties of the east-central part. The average yield of beans from this area is higher than from other areas of the State on soils of equal fertility. Experiments designed to increase the yield with commerical fertilizers have given inconsistent results. These experiments, carried out over a 21-year period by members of the Soil Science Section of the Michigan Agricultural Experiment Station, have shown a favorable early response of the crop to commercial fertilizer applications which would be maintained up to the blooming period, but often this apparent improvement in the growth of the crop was not reflected in the yield at harvest. In view of this situation, the following study was instituted in order to determine the effects of certain climatic factors on the development of the crop, especially during the critical period of pod formation. These experiments included a study of the effect of temperature, humidity, fertilizer, soil moisture, and leaf area on the set of pods and on the yield of the white pea bean both in the greenhouse and in the field.

PROCEDURE

The temperature and humidity measurements were secured from a Friez recording hygrothermograph which was placed at the level at which the plants were growing both in the greenhouse and in the field. Soil moisture was determined by drying the soil samples in an oven at 105° C. and calculating the percent of moisture from the resultant loss in weight.

GREENHOUSE EXPERIMENTS

In 1940 data on leaf area, yield, and set of pods were obtained from bean plants grown in 1-gallon jars in the greenhouse. The six treatments were as follows: A 4–16–8 fertilizer was applied to Miami loam at the rate of 600 pounds per acre at three soil-moisture levels, low, optimum, and high. The corresponding controls (low, optimum, and high moisture levels) received no fertilizer. The moisture levels were maintained by bringing the jars to original weight at frequent intervals. Two glass tubes, ¾ inch in diameter, were placed 1 inch and 5 inches, respectively, from the bottom of the jars. The capacity

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[2] The author wishes to express his appreciation to Dr. C. E. Millar and Dr. R. L. Cook for the valuable assistance and encouragement given throughout the duration of the project.

of these tubes was such that enough water could be added each day to keep the moisture content uniform throughout the jar. Three plants were grown in each jar and two replications of the six treatments were made. To extend the blooming period and so observe the plants under a greater variety of temperature and humidity conditions, the jars were planted at approximately 3-week intervals from April 6, 1940, to August 1, 1940. Whenever weather conditions permitted, the plants were removed to a wire-enclosed space adjacent to the greenhouse. The leaf area and yield of the individual plants were obtained, together with the set of pods and the temperature and humidity data as indicated by the hygrothermograph.

FIELD EXPERIMENTS

Field beans of the Michelite variety were planted in 28-inch rows in 1939, 1940, and 1941, on Miami loam at the Miller farm, Ovid, Mich. Plantings were made at approximately 5- to 7-day intervals for 6 weeks, from the last week in May to the first week in July. A 4-16-8 fertilizer at the rate of 500 pounds per acre was applied to one-half of the rows in a band 1 inch to the side of and 1¾ inches below the seed level. The hygrothermograph did not arrive in time to be used during 1939, and consequently only yield data from the different planting dates were taken in that year. In 1940 and 1941, just before blooming, plants were selected at random from the various treatments and the leaf area was determined according to the method described by Davis.[3] The number of leaf areas compiled depended on the amount of help available and the rapidity with which the plants came into bloom. The yields of both beans and straw of each plant was taken at harvesttime.

To determine the set of pods a small tag marked with the appropriate date was attached just below a pair of blossoms, and a few days later a count was made of the number of blossoms that formed pods.

Soil samples for moisture determination were collected at 2- to 3-day intervals throughout the blooming period.

In 1941 temperature and humidity data were taken at two locations in the bean-growing area, one of the locations supposedly more favorable for bean production than the other.

From the data taken the following correlations were made: Leaf area versus yield, yield of straw versus yield of beans, percent set of pods versus maximum temperature, percent set of pods versus minimum relative humidity, maximum temperature versus minimum relative humidity, percent soil moisture versus percent set of pods, percent set of pods versus maximum temperature with humidity held constant, percent set of pods versus minimum humidity with temperature held constant, and percent set of pods versus maximum temperature and minimum humidity combined. In addition, the effect of fertilizer on set of pods was calculated by analysis of variance, and the effect of date of planting and of fertilizer application on the yield of beans was obtained.

A prediction equation of temperature and yield was also calculated from the field data.

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FIELD EXPERIMENTS

RELATION BETWEEN SET OF PODS AND TEMPERATURE, SET OF PODS AND RELATIVE HUMIDITY, AND MAXIMUM TEMPERATURE AND MINIMUM RELATIVE HUMIDITY

The correlation coefficients and z values calculated for temperature and percent set of pods are reported in table 1. The values for r were calculated for six different factors, namely, percent set versus maximum temperature on day the blossoms were tagged; percent set versus average maximum temperature for the day the blossoms were tagged and for the following day; percent set versus the area under the curve as measured with a planimeter taken from the hygrothermograph chart with 60° and 70° used as bases for the measurements; the areas secured from both the 60° and 70° bases were correlated against percent set as area for the day the blossoms were tagged and also as the average area for the date the blossoms were tagged and the following day. The justification for using the area under the curve was based on the assumption that an intensity heat factor would be introduced which would result in a higher correlation between the percent set of pods than if maximum temperatures only were used. Since a relatively small number of items were correlated the r's were changed to z values [4] in order to determine whether or not any significant differences existed between the correlation coefficients calculated by the various methods. No significant differences were found, and the average maximum temperature for 2 successive days was later used in calculating the multiple and partial correlation coefficients.

The data in table 1 show a significant relation between the percent set of pods and minimum relative humidity.[5] However, minimum relative humidity does not exert as great an effect on the set of pods as does maximum temperature.

TABLE 1.—*Correlation coefficients and z values calculated between percent set of pods and maximum temperature, percent set of pods and minimum relative humidity, and maximum temperature and minimum relative humidity, Miller farm, 1940 and 1941*

Item	1940		1941		1940 and 1941	
	r	z value	r	z value	r	z value
Percent set of pods versus maximum temperature	−0.6107	−0.7100	−0.6576	−0.7886	−0.5962	−0.6872
Percent set of pods versus minimum relative humidity	.5126	.5662	.4698	.5098	.4653	.5042
Maximum temperature versus minimum relative humidity	−.4146	−.4417	−.4421	−.4747	−.4664	−.5055
Difference between z values required for significance		.8104		.7673		.5070

The correlation coefficients between maximum temperature and minimum relative humidity were calculated for each of the 2 years 1940 and 1941 and for the combined data for the 2 years. The average

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[5] Minimum relative humidity values for the date the blossoms were tagged were used in calculating the correlation coefficients. If areas under the curve are used, a negative correlation coefficient is obtained, making this method unsuitable.

maximum temperature for 2 successive days was correlated with the minimum relative humidity for the date the blossoms were tagged. The data show that a significant correlation of −0.4664 exists between maximum temperature and minimum relative humidity for the years 1940 and 1941. The degree of relationship between maximum temperature and minimum relative humidity is not as great as between the percent set of pods and maximum temperature.

MULTIPLE AND PARTIAL CORRELATIONS BETWEEN PERCENT SET OF PODS, MAXIMUM TEMPERATURE, AND MINIMUM RELATIVE HUMIDITY

The multiple and partial correlation coefficients between the percent set of pods, maximum temperature, and minimum relative humidity for 1940 and 1941 follow:

$Rx.yz$	0.6538
$Rxy.z$	−.5192
$Rxz.y$	.2523

x refers to the percent set of pods, y to the average maximum temperature for two successive days, and z to the minimum relative humidity for the date the blossoms were tagged.

The average maximum temperature for the 2 successive days and the minimum relative humidity for the date the blossoms were tagged were used in calculating both the multiple and the partial correlation coefficients. Two of the coefficients, $Rx.yz$ and $Rxy.z$, indicate a high degree of relationship between the factors mentioned. However, with temperature held constant the effect of humidity is not great enough to be significant, indicating that maximum temperature is the most important climatic factor affecting blossom development of the field bean.

PREDICTING PERCENT SET OF PODS FROM TEMPERATURE

A predicting line was calculated between percent set of pods and maximum temperature for 1940 and 1941. The equation follows:

Predicting equation	$y=-1.8x+192$
Standard error of prediction	3.82
Percent error of mean	7.62

x refers to the average maximum temperature for 2 successive days and y to the percent set of pods.

The equation indicates that approximately 57 percent of the blossoms will set pods if the average maxiumm temperature for any 2 sucessive days during the blooming period does not exceed 75° F. For each degree of temperature above 75° a reduction of approximately 2 percent in the set of pods will result. However, in using a predicting equation of this kind based on one climatic factor it should be remembered that there are a number of inherent errors that might be encountered for any specific time. For example, other climatic factors that influence the behavior of plants, such as soil temperature, soil moisture, relative humidity, and light intensity, may decrease the accuracy of a single prediction factor. Another important fact that must be considered is the difficulty of estimating the lag in the time required for a change in temperature to manifest itself in plant development. In working with beans it has been observed that after a few days of high temperature some time is required for the plants to recover sufficiently to take full advantage of a period of optimum temperature for set of pods. Another situation in which the

correlation between maximum temperature and percent set of pods is affected occurs when a day of optimum temperature comes during a period of high temperatures. In such cases the full advantage of an increase in the percent set of pods that would be expected if temperature were the only factor concerned is not attained.

In addition, strict linearity of the data is assumed, and in this respect it should be pointed out that this equation is applicable only within the range of temperature of 55° to 98° encountered in this investigation. Predicting the percent set of pods from temperatures outside this range could easily lead to erroneous results.

TEMPERATURE AND RELATIVE HUMIDITY VARIATIONS WITH RESPECT TO LOCALITY

The data in table 2 show that during the blossoming period of 1941 the average maximum daily temperature was significantly lower and the average minimum daily relative humidity significantly higher at the Horst farm, located in the best bean-growing area, than at the Miller farm, located just outside of this area. Although these data are for only one year, they are based on daily records for two different periods, from July 14 to August 31 and from July 14 to August 17. The July 14–August 17 period is the time in which most of the pods are formed, and comparisons made between temperature and humidity conditions at the two locations during this critical period should give more practical information regarding climatic effects than if the data for the July 14–August 31 period alone were used. The mean differences for both temperature and minimum humidity for the Miller farm are significant at the 1-percent level in all cases except one, that is, the mean difference for relative humidity for the July 14–August 31 period, and in this case the measure of significance is well above the 5-percent level. Since it has been shown that after the maximum temperature has reached 75° F. the percent set of pods rapidly decreases and that there is also some relation between percent set of pods and minimum relative humidity, it follows that an area in which lower maximum daily temperature and higher minimum relative humidity prevail would be more favorable for this crop provided soil conditions are comparable. The assumption is borne out by the facts of the case since the Horst farm is in the center of the best bean-growing area in the State and the Miller farm is outside of this area.

TABLE 2.—*Mean differences between daily maximum temperature and minimum relative humidity at the Miller and Horst farms in 1941*

Location[1]	Maximum temperature				Minimum relative humidity			
	July 14–Aug. 31		July 14–Aug. 17		July 14–Aug. 31		July 14–Aug. 17	
	Mean	Mean difference	Mean	Mean difference	Mean	Mean difference	Mean	Mean difference
Miller farm	80.43	3.79*	82.17	3.54*	29.86	2.46**	27.83	3.54*
Horst farm	76.64		78.63		32.32		31.37	

[1] The Horst farm at Akron, Mich., is located in the most favorable bean-growing area and the Miller farm at Ovid, just outside of this area.

* Significant at the 1-percent level.

** Significant at the 5-percent level.

INFLUENCE OF SOIL MOISTURE ON SET OF PODS

The correlation coefficients calculated between percent set of pods and soil moisture for the Miller farm for 1940 and 1941 are shown in the following tabulation:

Percent set of pods versus percent moisture in surface soil..... [1] 0.3482
Percent set of pods versus percent moisture in subsoil..... .1913

According to these data neither the percent of moisture in the surface 6 inches of soil nor in the subsoil had any significant effect on set of pods.

INFLUENCE OF FERTILIZER ON SET OF PODS

To determine the effect of fertilizer on set of pods a 4–16–8 fertilizer was applied at the rate of 500 pounds per acre in a band 1 inch to the side and 1¾ inches below the seed in 1940 and 1941. The results, presented in table 3, show no apparent influence of fertilizer on set of pods in either season. However, the difference in the percent set of pods on the various dates was highly significant, indicating again the role of climatic factors in the production of the field bean. Table 4 presents the results of an analysis of the variance of yield of beans, as shown in table 3.

TABLE 3.—*Effect of fertilizer on set of pods, Miller farm,*[1] *1940 and 1941*

Percent set in 1940				Percent set in 1941			
Date	Fertilized	Unfertilized	Weighted mean [2]	Date	Fertilized	Unfertilized	Weighted mean [2]
July 23	10.0	2.5	3.5	July 14	67.1	52.6	60.2
July 24	10.6	6.7	8.4	July 16	80.1	69.3	74.4
July 26	18.8	12.1	15.8	July 18	91.8	85.9	89.7
July 29	11.1	12.4	11.8	July 21	78.9	71.2	74.1
July 30	5.1	6.5	5.7	July 23	75.5	65.6	69.8
August 2	22.1	24.7	23.2	July 25	38.6	27.3	32.9
August 5	50.7	49.7	50.2	July 28	19.7	18.9	19.5
August 7	51.8	67.6	59.3	July 30	39.1	23.9	36.5
August 10	72.5	74.8	73.5	August 1	21.7	25.7	22.8
August 12	74.1	72.5	73.4	August 4	13.0	14.6	13.5
August 14	46.3	54.3	51.9	August 6	4.3	15.0	9.0
August 16	73.9	81.3	77.1	August 8	16.2	20.1	13.6
August 19	63.3	64.0	63.6	August 11	53.9	55.5	52.4
August 20	68.4	76.5	73.8	August 13	29.1	50.0	37.2
August 23	53.3	78.9	66.3	August 25	72.8	73.4	73.2
August 31	89.8	84.8	87.3	August 28	73.1	58.3	72.2
September 3	94.0	92.3	93.1				

[1] The percent set of pods is based on 21,036 blossoms in 1940 and 14,508 in 1941, a total of 35,544 blossoms for the 2 years.
[2] Means weighted according to number of blossoms counted.

TABLE 4.—*Analysis of variance of set of pods shown in Table 3*

1940				1941			
Source of variance	Degrees of freedom	Sum of squares	Mean square	Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	33	30,817.24		Total	31	21,339.30	
Dates	16	30,190.56	1,886.91*	Dates	15	20,496.60	1,366.44*
Fertilizer	1	61.70	61.70	Fertilizer	1	70.81	70.81
Error	16	565.18	35.51	Error	15	771.89	51.46

*Significant at the 1-percent level.

[1] A value of 0.3976 is required for significance at the 5-percent level.

INFLUENCE OF FERTILIZER AND DATE OF PLANTING ON YIELD

The data in table 5 show that a 4–16–8 fertilizer applied at the rate of 500 pounds per acre to a bean crop in 1939 significantly increased the yield regardless of the planting date and that the yields for both the earliest and latest planting dates, May 31 and June 12, respectively, were significantly higher than those from either of the plantings made on June 3 or June 9. The yield from the planting made on June 6 was not significantly higher or lower than any of the other yields. The lack of any definite trend in yields and planting dates, although there is a direct relation between date of planting and time of blossoming, further indicates the possibility that differences in yield from different dates of planting are due to the weather conditions prevailing during the blooming period. Table 6 presents the results of an analysis of the variance of yield of beans, as shown in table 5.

TABLE 5.—*Effect of fertilizer and date of planting on yield of beans, Miller farm, 1939*

Planting date	Yield per acre [1]		Planting date	Yield per acre [1]	
	Fertilized [2]	Unfertilized		Fertilized [2]	Unfertilized
	Bushels	*Bushels*		*Bushels*	*Bushels*
May 31	14.0	8.9	June 9	10.3	5.6
June 3	10.4	6.4	June 12	12.7	7.0
June 6	11.9	6.3			

[1] Average of 4 replications.
[2] Fertilized at rate of 500 pounds of 4–16–8 fertilizer per acre.

TABLE 6.—*Analysis of variance of yield of beans, as shown in table 5*

Source of Variance	Degrees of freedom	Sum of squares	Mean square	Source of variance	Degrees of freedom	Sum of squares	Mean square
Total	39	533.77		Fertilizer	1	248.56	248.56**
Blocks	3	117.65	39.21	Fertilizer × dates	4	3.94	.99
Dates	4	60.58	15.14*	Error (b)	19	50.24	2.64
Blocks × dates (a)	12	52.80	4.40				

* Significant at the 5-percent level.
** Significant at the 1-percent level.

INFLUENCE OF FERTILIZER ON LEAF AREA AND YIELD OF BEANS AND STRAW

The data in table 7 show that a 4–16–8 fertilizer applied at the rate of 500 pounds per acre caused a significant increase both in leaf area and in yield of straw and beans in some cases, but these increases were more consistent in 1941 than in 1940. In 1940, in only one case in five did a significant increase in either the yield of beans or of straw result from an application of fertilizer. However, in three cases in five a significant increase in leaf area was observed. In all cases in 1941 significant increases in yield of straw and in leaf area resulted from a fertilizer application, and in three out of four cases the yield of beans was significantly better. These data indicate a considerable seasonal effect of fertilizer on plant behavior. It is interesting to note that the significant differences found in 1940 were significant only at the 5-percent level, whereas in 1941 all significant

differences were at the 1-percent level. This situation may be partly due to the greater competition between plants in 1940 resulting from closer spacing in that year. In 1940 the plants were thinned to approximately 4 inches apart and in 1941 to 8 inches apart.

The effect of date of planting on the leaf area and on the yield of both straw and beans is noted in the wide variation between the values for the different planting dates. In 1940 the leaf area ranged from 201.2 to 474.4 square inches for plants receiving fertilizer and from 154.9 to 406.2 square inches for plants not receiving fertilizer. Similar variation in both the yield of beans and straw were found. The variations in leaf area and in yields of beans were not as wide in 1941 as in 1940, again emphasizing the effect of the season.

TABLE 7.—*Mean differences of yield of beans, leaf area, and yield of straw between fertilized and unfertilized bean plants, Miller farm, 1940 and 1941* [1]

Date planted	Yield of beans	Leaf area	Yield of straw
	1940		
	Grams	*Sq. In.*	*Grams*
June 8	5.1±9.4	54.5±33.6	4.6±6.2
June 11	−1.7±6.1	71.6±22.5*	3.6±4.3
June 14	18.1±5.7*	83.1±20.0*	12.9±4.7*
June 17	−3.0±4.9	46.3±14.8*	−1.7±2.8
June 25	3.7±4.3	68.2±48.5	6.0±4.1
	1941		
June 3	7.5±6.7	166.0±33.9**	28.7±4.9**
June 9	13.4±2.2**	163.9±23.5**	17.3±2.3**
June 16	9.8±2.2**	163.7±26.4**	11.9±1.8**
June 24	7.6±1.9**	142.2±19.8**	9.3±1.6**

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[1] A minus sign preceding a yield figure indicates that yield of fertilized plants was below that of unfertilized plants.

LEAF AREA AND YIELD RELATIONSHIPS

The data in tables 8 and 9 show the relation between leaf area and yield of beans and between weight of straw and yield of beans for individual plants grown in the field in 1940 and 1941. The correlation coefficients for the various planting dates may be either significantly positive or significantly negative, depending on weather conditions during the blossoming period. These coefficients show that little correlation exists between leaf area and yield of the field bean. The relation between yield of beans and yield of straw is, on the other hand, is highly significant for each planting date in 1940 and for five out of eight in 1941, indicating that the weight of straw is a better measurement of the yield of beans than the leaf area.

Fertilizer does not seem to have any consistent effect on the relation of leaf area and yield of beans or weight of straw and yield of beans.

TABLE 8.—*Correlation coefficients calculated between leaf area versus yield of beans and weight of straw versus yield of beans, Miller farm, 1940*

Date of planting	Date set of pods was determined	Leaf area versus yield of beans	Weight of straw versus yield of beans
FERTILIZED [1]			
June 8	July 16–Aug. 14	0.5522*	0.9403**
June 11	July 22–Aug. 14	.3726	.7859**
June 14	July 30–Aug. 16	−.7366*	.8989**
June 17	July 30–Aug. 20	−.2746	.9121**
June 25	Aug. 16–Aug. 23	.4500	.6703**
UNFERTILIZED			
June 8	July 23–Aug. 14	0.6044*	0.9674**
June 11	July 24–Aug. 19	.3853	.8693**
June 14	July 29–Aug. 12	.3127	.7867**
June 17	July 30–Aug. 20	.2922	.6733**
June 25	July 16–Aug. 23	.2800	.6474**

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[1] Bean plants were fertilized at the rate of 500 pounds per acre of 4–16–8 fertilizer.

TABLE 9.—*Correlation coefficients calculated between leaf area versus yield of beans and weight of straw versus yield of beans, Miller farm, 1941*

Date of planting	Date set of pods was determined	Leaf area versus yield of beans	Weight of straw versus yield of beans
FERTILIZED [1]			
June 3	July 14–July 28	0.5381	0.0579
June 9	July 23–Aug. 13	.5015*	.1732
June 16	July 30–Aug. 11	.5488*	.5474*
June 24	Aug. 22–Aug. 28	.1539	.6169**
UNFERTILIZED			
June 3	July 14–July 30	0.5843	0.3956
June 9	July 30–Aug. 13	.4526*	.8850**
June 16	Aug. 6–Aug. 11	.2026	.6077**
June 24	Aug. 22–Aug. 28	.4735*	.7969**

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[1] Bean plants were fertilized at the rate of 500 pounds per acre of 4–16–8 fertilizer.

GREENHOUSE EXPERIMENTS

The results obtained in the greenhouse differ in some respects from those obtained in the field. These differences may be briefly summarized as follows: Individual plants grown in the field had on an average 31 times more beans and 7 times more leaf area than plants grown in the greenhouse, and the ratio of beans to straw varied widely in plants grown under greenhouse and field conditions. Contrary to the results obtained in the field, no significant correlation was found between maximum temperature and percent set of pods or minimum relative humidity and percent set of pods in the greenhouse. However, the correlation between maximum temperature and minimum relative humidity under greenhouse conditions was found to be significant. In view of the results of this study, the assumption that

results obtained in the greenhouse in an investigation of this nature are applicable under field conditions is open to question.

DISCUSSION OF RESULTS

Among the factors that influence the development of the field bean plant, in addition to those studied, are wind velocity, amount, distribution, and intensity of rainfall, degree of cloudiness, and plant cover. These factors are especially important during the blossoming stage. A short discussion of the possible ways in which they may influence the bean plant, together with a few remarks on the growth habit of the plant, may help to explain some of the results obtained.

The recommended planting date for beans in Michigan is from June 1 to 10, and harvest begins approximately 3 months later. Blossoming begins 6 to 7 weeks after planting, starts at the lower part of the plant, progresses toward the tips of the branches, and extends to the runners if weather conditions are favorable. The plants may blossom, set pods, and continue to bloom for as long as 5 or 6 weeks if a wet period occurs late in the season. Thus there are ripe pods present and blossoms forming at the same time. In case of an early frost many of the immature pods formed during this late blooming period will be damaged and the quality of the crop will be appreciably lowered. However, the length of the blooming period makes it possible to get a fair yield even though weather conditions during part of this period are unfavorable.

It has often been observed that during days of high temperature and low relative humidity, if the wind velocity is high, a much lower set of pods is obtained than under similar conditions of temperature and humidity with wind velocity low. In fact, farmers associate these hot, dry winds with blasting of the blossoms. Wind velocity, then, can be a contributing factor in the set of pods and thus modify the effect of temperature alone.

Rainfall is also an important factor as, in addition to supplying soil moisture, it exerts a mechanical effect on blossom development. During heavy rains, the blossoms on the tips of branches or runners are driven into the ground and thus the set of pods is reduced. When the surface of the soil remains moist for long periods many of the blossoms that touch the ground rot and thus the set of pods is further reduced. It is apparent, therefore, that intensity and amount of rainfall affect the percent of pods independently of temperature, and so reduce the degree of correlation between temperature and set of pods.

The degree of cloudiness exerts a slight effect on the set of pods by modifying the effect of temperature and light. For example, if the maximum temperature is used in correlating temperature and percent set of pods, then during a cloudy day the length of time that the maximum temperature would affect the plant would be less than during a clear day with the same temperature, and the percent set of pods for each day would be different.

Temperature is the climatic factor that exerts the strongest influence on the percent set of pods. According to the results obtained, a significant correlation exists between these two factors, and the percent set of pods can be predicted with a fair degree of accuracy from maximum temperatures. The error for any single predicted value is 7.6 percent of the mean. This error is quite large, but in

consideration of the fact that temperature is only one of the factors involved, the magnitude of the error is well within the expected. This demonstrated effect of temperature shows the practicality of this type of study for determining whether or not any particular location would be suitable for the production of field beans. Significant daily temperature differences found in 1941 between two farms, one located in the typical bean area and the other just outside this area, add considerable weight to the data since here is a practical demonstration of conditions actually existing in the field for which a plausible explanation can be offered.

The inconsistency of annual response of the field bean to fertilizer can be explained largely as a result of the influence of temperature.

FIGURE 1.—The bean plants on the left were planted 1 week earlier than those on the right and blossomed during a more favorable period for setting pods. The plants on the left are well podded, whereas those on the right are practically devoid of pods.

In spite of the fact that the fertilizer stimulates vegetative growth, yield will be poor unless the temperatures prevailing during the blooming period are favorable. It has often been observed that fertilizer will hasten the blooming period just enough to cause the majority of blossoms to form during a hot, dry period and consequently there will be no increase in yield from the use of the fertilizer. The effect of date of planting on yield is illustrated by the plants shown in figure 1. There was just 1 week's difference in the date of planting, yet the plants in one case are well podded and in the other they are practically devoid of pods.

As compared with the effect of maximum temperature on the percent set of pods the effect of minimum relative humidity is of minor

importance, although a significant correlation was found between these two factors. In general, high maximum temperature is associated with low relative humidity, but this association was not found to be significant under the field conditions encountered except when the 2 years' data were combined. However, it was found that the average minimum relative humidity during the blooming period was significantly higher on a farm located in the typical bean-growing area than on another farm outside of this area, which suggests that humidity might have influenced the results.

Set of pods was not found to be associated to any appreciable degree with soil moisture changes. However, the data should be interpreted as meaning that the amount of moisture found in the soil did not reach a critical point either from the standpoint of an excess or a too limited supply. In other words, the data apply only within the limits encountered in the experiment because it is selfevident that any portion of a plant will not develop naturally if there is either an excess or a deficiency of moisture.

In studies of this nature, it is essential that the weather-recording instruments be placed in the area in which the experiment is being conducted and at the same level as that at which the plants are growing. Weather data supplied by a weather station even in the general vicinity cannot be depended upon to give a true picture of conditions prevailing at the experimental location. The modifying effect of the plant cover is not accounted for in weather data obtained in other than the same location as that in which the experimental plants are growing.

It might be assumed that the extent of leaf area of a plant would influence the yield since the ability of a plant to manufacture food increases with greater leaf area. However, the data obtained in this study do not show that this relationship is at all constant. Depending on the weather conditions at the time of pod formation, the correlation between leaf area and yield may be either positive or negative. This situation, then, implies the existence of other factors that influence the ratio of the yield of seed to leaf area. As the plants were grown within a limited area of the same soil, thus excluding the factor of soil fertility, the factors remaining must of necessity be climatic. The data show the temperature during the blossoming period to be the most important climatic factor involved.

SUMMARY AND CONCLUSIONS

The effects of temperature, humidity, soil moisture, leaf area, and fertilizer on the behavior of the white pea bean (*Phaseolus vulgaris* L.) were investigated both in the greenhouse and in the field. From the field studies the following conclusions were reached:

(1) Maximum temperature influences the set of pods more than any other of the factors studied and the percent set of pods can be predicted from maximum temperature with a fair degree of accuracy.

(2) Minimum relative humidity and soil moisture, within the limits encountered in the work, exert only a minor influence on the set of pods.

(3) The relation of leaf area to yield of beans may range from a positive to a negative correlation depending on the weather pre-

vailing during the blooming period. The yield of beans therefore, cannot be safely predicted from leaf area.

(4) Fertilizer had no effect on set of pods.

The response to environmental factors of plants grown under greenhouse conditions differed in certain important respects from that of plants grown in the field.

A study of the nature of the one herein reported is valuable for determining what areas may be best suited for crops that, like the white pea bean, have critical environmental requirements.

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